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Phenolic Compounds from *Scorzonera tomentosa* L.

Sarı, A., Zidorn, C., Ellmerer, E.P., **Özgökçe, F.,** Ongania KH., Stuppner, H.

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Phenolic Compounds from *Scorzonera tomentosa* L.

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Abstract

From the subaerial parts of *S. tomentosa* L. (Asteraceae), two new dihydroisocoumarins, compounds **1** and **2**, a new phthalide, **3**, and a new stilbene derivative, **4**, were isolated, together with four known compounds, (±)-hydrangenol (**5**), (−)-hydrangenol 4′-*O*-*β*-glucoside (**6**), (±)-hydramacrophyllol A (**7**), and (±)-hydramacrophyllol B (**8**). All secondary metabolites were identified on the basis of physicochemical, spectroscopic, and mass-spectrometric data. The known compounds **5**–**8** were isolated for the first time from this species.

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Phenolic Compounds from *Scorzonera tomentosa* L.

by **Aynur Sarı***[a]), **Christian Zidorn**[b]), **Ernst P. Ellmerer**[c]), **Fevzi Özgökçe**[d]), **Karl-Hans Ongania**[c]), and **Hermann Stuppner**[b])

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[d]) Yüzüncü Yıl University, Faculty of Sciences and Letters, Department of Biology, 65180 Van, Turkey

From the subaerial parts of *S. tomentosa* L. (Asteraceae), two new dihydroisocoumarins, compounds **1** and **2**, a new phthalide, **3**, and a new stilbene derivative, **4**, were isolated, together with four known compounds, (±)-hydrangenol (**5**), (−)-hydrangenol 4′-*O*-*β*-glucoside (**6**), (±)-hydramacrophyllol A (**7**), and (±)-hydramacrophyllol B (**8**). All secondary metabolites were identified on the basis of physicochemical, spectroscopic, and mass-spectrometric data. The known compounds **5**–**8** were isolated for the first time from this species.

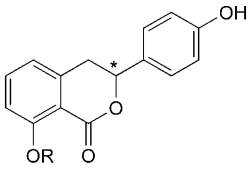
Introduction. – The genus *Scorzonera* is a member of the family Asteraceae, which is a known source of numerous classes of bioactive natural products [1]. For the European flora, some 28 *Scorzonera* species have been reported [2], and the flora of Turkey encompasses 39 species, including 17 endemics [3]. Previous chemical investigations of this genus yielded dihydroisocoumarins, flavonoids, lignans, phenolic acids, a sesquiterpene, sesquiterpene lactones, triterpenes, and a new class of bibenzyl derivatives [4–17].

Scorzonera tomentosa L. is a perennial herb endemic to Turkey [3]. Its subaerial parts are used in traditional medicine as analgesic, antirheumatic (in the form of plaster), and anthelmintic, as well as for the treatment of infertility [18][19]. In a previous paper [17], the isolation of stigmasterol 3*β*-glucoside, *β*-sitosterol, lupeol, lupeol acetate, and *α*-amyrine from the aerial parts of *S. tomentosa* was reported. In the current study, we report four new constituents, compounds **1**–**4**, from the subaerial parts of this plant, together with four known compounds: (±)-hydrangenol (**5**), (−)-hydrangenol 4′-*O*-glucoside (**6**), and (±)-hydramacrophyllol A (**7**) and B (**8**).

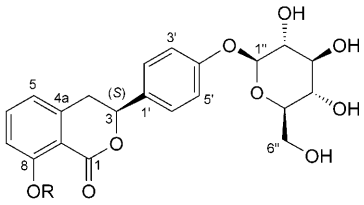
Results and Discussion. – The AcOEt-soluble fraction of the MeOH extract of the subaerial parts of *S. tomentosa* L. was investigated to elucidate its secondary-metabolite profile.

Compound **1** was isolated as a colorless, optically inactive powder ($[\alpha]_D^{25} = 0$). The UV spectrum of **1** showed absorption maxima at 246 (log $\varepsilon = 4.1$) and 306 (3.9) nm. The IR spectrum showed absorption bands due to phenolic OH (3450), C=O (1736), and aliphatic groups (3063 cm^{-1}), as well as due to an aromatic ring (1600, 1584, 1475 cm^{-1}). In the ESI mass spectrum, the $[M-H]^-$ signal was observed at *m*/*z* 269, consis-

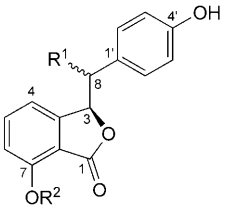
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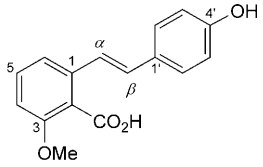
(±)-**1** R = Me
(±)-**5** R = H



(–)-**2** R = Me
(–)-**6** R = H



(±)-**3** R^1 = α-OH, R^2 = Me
(±)-**7** R^1 = α-OH, R^2 = H
(±)-**8** R^1 = β-OH, R^2 = H



4

tent with the molecular formula $C_{16}H_{14}O_4$. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed the signals ascribable to a 1,4-disubstituted benzene ring [δ(H) 7.32 (*d*, *J* = 8.5 Hz, 2 H); 6.81 (*d*, *J* = 8.5 Hz, 2 H)], a trisubstituted benzene ring [δ(H) 6.94 (*d*, *J* = 7.5 Hz, 1 H); 7.56 (*t*, *J* = 7.5 Hz, 1 H); 7.08 (*d*, *J* = 8.5 Hz, 1 H)], a δ-lactone [δ(H) 5.40 (*dd*, *J* = 3.0, 12.0 Hz, 1 H); 3.03 (*dd*, *J* = 3.0, 16.0 Hz, 1 H); 3.26–3.36 (*m*, 1 H)], and a MeO group [δ(H) 3.91 (*s*, 3 H)].

The ^{13}C-NMR spectrum of **1** (*Table 2*) revealed the presence of twelve aromatic C-atoms (three quaternary, two oxygenated quaternary, and seven CH), as well as one CH_2, one oxygenated CH, one MeO, and one C=O group. The position of the MeO group was established at C(8) from the ^{1}H-NMR coupling pattern, and corroborated by HMBC experiments (*Table 3*). In the HMBC spectrum, H–C(5) showed a strong three-bond correlation with C(4) at δ(C) 37.05, and the MeO H-atoms at δ(H) 3.91 were correlated with C(8) at δ(C) 162.6. From these data, the structure of racemic **1** was established as (3*RS*)-3,4-dihydro-3-(4-hydroxyphenyl)-8-methoxy-1*H*-2-benzopyran-1-one, and named *(±)-scorzotomentosin*.

Compound **2** was isolated as a colorless powder. HR-FAB-MS displayed signals at *m*/*z* 455.1325 ($[M+Na]^+$; calc. 455.1318) and 433.1507 ($[M+H]^+$, calc. 433.1499), consistent with the molecular formula $C_{22}H_{24}O_9$. The data observed in the ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*, resp.) were very similar to those of **1**, but further indicated the presence of a sugar moiety. The anomeric H-atom at δ(H) 4.94 (*d*, *J* = 7.0 Hz) and the coupling pattern of the other sugar resonances indicated a β-glucose (Glc) unit. The position of the glucoside linkage was established by a HMBC experiment (*Table 3*), which revealed correlations between the anomeric H-atom, H–

Table 1. *^{1}H-NMR Spectroscopic Data of* **1**–**4**. At 500 MHz in CD_3OD (**1**–**3**) or (D_6)DMSO (**4**); δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**1**	**2**[a])	**3**	**4**
3	5.40 (*dd*, *J* = 3.0, 12.0)	5.46 (*dd*, *J* = 3.0, 12.0)	5.63 (*d*, *J* = 4.0)	–
4	3.03 (*dd*, *J* = 3.0, 16.0) 3.26–3.36 (*m*)	3.17 (*dd*, *J* = 3.0, 16.0) 3.26–3.36 (*m*)	6.73 (*d*, *J* = 8.0)	6.77 (*d*, *J* = 8.0)
5	6.94 (*d*, *J* = 7.5)	6.94 (*d*, *J* = 7.5)	7.53 (*dd*, *J* = 7.5)	7.10 (*dd*, *J* = 7.5, 8.0)
6	7.56 (*t*, *J* = 7.5)	7.56 (*t*, *J* = 8.5)	7.00 (*d*, *J* = 8.5)	7.26 (*d*, *J* = 8.0)
7	7.08 (*d*, *J* = 8.5)	7.08 (*d*, *J* = 8.5)	–	–
8	–	–	5.03 (*d*, *J* = 4.0)	–
2′,6′	7.32 (*d*, *J* = 8.5)	7.44 (*d*, *J* = 8.5)	7.12 (*d*, *J* = 8.5)	7.34 (*d*, *J* = 8.5)
3′,5′	6.81 (*d*, *J* = 8.5)	7.14 (*d*, *J* = 8.5)	6.68 (*d*, *J* = 8.5)	6.82 (*d*, *J* = 8.5)
α	–	–	–	7.13 (*d*, *J* = 16.0)
β	–	–	–	7.03 (*d*, *J* = 16.0)
MeO	3.91 (*s*)	3.91 (*s*)	3.91 (*s*)	3.71 (*s*)

[a]) Sugar resonances: δ(H) 4.94 (*d*, *J* = 7.0, H–C(1″)); 3.48 (*dd*, *J* = 9.0, 7.0, H–C(2″)); 3.45 (*t*, *J* = 9.0, H–C(3″)); 3.40 (*t*, *J* = 9.0, H–C(4″)); 3.42–3.50 (*m*, H–C(5″)); 3.70 (*dd*, *J* = 12.0, 5.0, 1 H of CH_2(6″)); 3.90 (*dd*, *J* = 12.0, 2.0, 1 H of CH_2(6″)).

Table 2. *^{13}C-NMR Spectroscopic Data of* **1**–**4**. At 125 MHz in CD_3OD (**1**–**3**) or (D_6)DMSO (**4**); δ in ppm; signal assignment by HSQC and HMBC experiments. Arbitrary atom numbering.

Position	**1**	**2**	**3**	**4**	Position	**1**	**2**	**3**	**4**
1	165.5	164.0	170.0	133.1	3′	116.3	117.5	115.0	115.4
2	–	–	–	115.7	4′	159.0	158.0	158.5	158.2
3	81.0	80.5	85.2	154.6	5′	116.3	117.5	115.0	115.4
3a	–	–	150.5	–	6′	128.9	128.4	128.5	127.5
4	37.05	36.0	116.5	109.1	α	–	–	–	123.6
4a	143.9	142.5	–	–	β	–	–	–	128.0
5	120.6	120.5	136.5	126.0	1″	–	102.0	–	–
6	136.4	136.5	112.0	115.7	2″	–	74.6	–	–
7	112.3	112.0	159.0	–	3″	–	77.9	–	–
7a	–	–	115.0	–	4″	–	71.1	–	–
8	162.6	162.0	76.0	–	5″	–	77.7	–	–
8a	114.3	113.5	–	–	6″	–	61.2	–	–
1′	130.8	133.0	129.0	128.0	MeO	56.4	56.2	55.2	54.5
2′	128.9	128.4	128.5	127.5	COOH	–	–	–	172.1

C(1″), and the quaternary C-atom at δ(C) 158.0 (C(4′))[1]). Consequently, **2** was identified as *(−)-scorzotomentosin 4′*-O-*β-glucoside.*

The absolute configuration at C(3) was determined by comparison of the CD spectrum of **2** with that of scorzocreticin [9]. Compound **2** gave rise to a positive *Cotton* effect at 241 nm, and a negative one at 263 nm, as in the case of scorzocreticin. This suggested that both compounds have the absolute (*S*)-configuration at C(3).

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[1]) Arbitrary atom numbering (see chemical formulae).

Table 3. *Key HMBC Cross-Peaks for* **1** *and* **2**

H-Atom	C-Atom	
	1	**2**
H–C(3)	C(1), C(4a), C(2′,6′)	C(1), C(4a), C(2′,6′)
H–C(4)	C(4a), C(5), C(8a), C(1′)	C(4a), C(5), C(8a), C(1′)
H–C(5)	C(4), C(7), C(8a)	C(4), C(7), C(8a)
H–C(6)	C(4a), C(8)	C(4a), C(8)
H–C(7)	C(5), C(8a)	C(5), C(8a)
H–C(2′,6′)	C(3), C(2′,6′), C(4′)	C(3), C(2′,6′), C(4′)
H–C(3′,5′)	C(1′), C(3′,5′)	C(1′), C(3′/5′)
MeO	C(8)	C(8)
H–C(1″)	–	C(4′)

Compound **3**, a colorless, optically inactive powder, showed UV absorbances at 228 ($\log \varepsilon = 4.2$), 285 (3.6), and 301 (3.7) nm. Its IR spectrum indicated phenolic OH (3440), C=O (1740), and aryl (1620, 1607, 1470 cm^{-1}) groups. The molecular formula $C_{16}H_{14}O_5$ was determined by HR-FAB-MS (*m*/*z* 287.0912 ($[M+H]^+$; calc. 287.0919)). The ^{1}H-NMR spectrum of **3** (*Table 1*) was similar to that of hydramacrophyllol B (**8**) [20], with a 1,2,3-trisubstituted benzene ring (δ(H) 6.73 (*d*, $J = 8.0$ Hz, 1 H); 7.53 (*dd*, $J = 7.5$ Hz, 1 H); 7.00 (*d*, $J = 8.5$ Hz, 1 H)), a 1,4-disubstituted aromatic ring [δ(H) 7.12, 6.68 (2*d*, $J = 8.5$ Hz each, 2×2 H)]. Furthermore, the signals of a CH adjacent to an OH group, and those of a CH adjacent to a lactone O-atom were observed (δ(H) 5.03 (*d*, $J = 4.0$ Hz, 1 H); 5.63 (*d*, $J = 4.0$ Hz, 1 H)). Finally, a MeO group resonated at δ(H) 3.91 (*s*).

In the ^{13}C-NMR spectrum of **3** (*Table 2*), twelve aromatic C-atoms (three quaternary, two oxygenated quaternary, and seven CH), two oxygenated CH groups, one MeO, and one C=O group were observed. The MeO group was placed at C(7), as deduced from the ^{1}H-NMR coupling pattern and interpretation of the HMBC spectrum (*Table 4*). In the latter, H–C(4) at δ(H) 6.73 showed a strong three-bond correlation with C(3) at δ(C) 85.2, and the MeO H-atoms at δ(H) 3.91 were correlated with C(7) at δ(C) 159.0. The configurations at C(3) and C(8) were deduced from the NMR data in comparison to those reported for **8** [20]. Thus, from the above data, the structure of racemic **3** was identified as (3*RS*)-3-[(*SR*)-hydroxy(4-hydroxyphenyl)-methyl]-7-methoxy-2-benzofuran-1(3*H*)-one, and named *(±)-scorzophthalide*.

Compound **4** was isolated as a yellowish powder. Its IR spectrum showed absorptions typical of OH (3440), C=O (1660), and benzene-ring moieties (1590, 1515, 1460, 1400 cm^{-1}). The UV spectrum was characteristic for a stilbene derivative [21], with absorption maxima at 217 ($\log \varepsilon = 4.2$), 306 (4.31), and 320 (4.32) nm. The ESI mass spectrum of **4** displayed the $[M-H]^-$ ion at *m*/*z* 269, consistent with the molecular formula $C_{16}H_{14}O_4$. The ^{1}H-NMR spectrum (*Table 1*) indicated a 1,4-disubstituted benzene ring, a trisubstituted benzene ring, a MeO group, and two vinylic H-atoms [δ(H) 7.03, 7.13 (2*d*, $J = 16.0$ Hz, 1 H each)], which were assigned to an (*E*)-stilbene. The ^{13}C-NMR spectrum (*Table 2*) indicated 16 C-atoms, including one MeO, nine CH, three quaternary C-atoms, two oxygenated aryl C-atoms, and one COOH group. HMBC experiments (*Table 4*) showed long-range correlations between the MeO H-atoms at

Table 4. *Key HMBC Cross-Peaks for* **3** *and* **4**

H-Atom	C-Atom	
	3	**4**
H–C(3)	C(1), C(4), C(7a), C(1′)	–
H–C(4)	C(3), C(6), C(7a)	C(2), C(6)
H–C(5)	C(3a), C(7)	C(1), C(3), C(4)
H–C(6)	C(4), C(7a)	C(2), C(4), C(α)
H–C(8)	C(3a), C(2′,6′)	–
H–C(*α*)	–	C(1), C(2), C(6), C(1′)
H–C(*β*)	–	C(1), C(2′,6′), C(α)
H–C(2′,6′)	C(4′), C(2′,6′), C(8)	C(4′), C(2′,6′), C(*β*)
H–C(3′,5′)	C(1′), C(3′,5′)	C(1′), C(3′,5′)
MeO	C(7)	C(3)

δ(H) 3.71 and the quaternary C-atom at δ(C) 154.6 (C(3)), and also between the aromatic resonance at δ(H) 7.10 (H–C(5)) and the two quaternary C-atoms at δ(C) 133.1 (C(1)) and 154.6 (C(3)). Hence, the MeO group was placed at C(3), and the COOH group was attached at C(2). From these data, the structure of **4** was determined as 2-[(*E*)-2-(4-hydroxyphenyl)ethenyl]-6-methoxybenzoic acid, and named *scorzoerzincanin*.

The four known compounds, **5**–**8**, had been isolated before from the genus *Hydrangea*, and were isolated from *S. tomentosa* for the first time. They were identified on the basis of their physicochemical and spectroscopic data [20–24].

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Experimental Part

General. Column chromatography (CC): silica gel *60* (40–63 μm; *Merck*) or *Sephadex LH-20* (*Sigma-Aldrich*). Prep. TLC: precoated silica gel *60* F_{254} plates (0.25 mm; *Merck*). Semi-prep. HPLC: injection of 40-μl aliquots of sample soln. (25 mg/ml) on a *Merck RP-18* column (*LiChrosphere*, 250×10 mm, 10 μm) at a column temp. of 55°; isocratic MeCN/H_2O flow at 2 ml/min; stop time: 45 min, post time: 15 min; detection at 205 nm. UV Spectra: *Jasco V-530* spectrophotometer, in MeOH; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 552* spectrophotometer, KBr cells; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Varian UnityPlus-500* spectrometer, at 500/125 MHz, resp., in CD_3OD or $(CD_3)_2SO$; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *Finnigan MAT SSQ-7000* mass spectrometer. HR-FAB-MS: *Finnigan MAT-95* mass spectrometer, glycerol as matrix; in *m*/*z*.

Plant Material. *Scorzonera tomentosa* L. was collected from Erzincan, East Anatolia, at an altitude of 1560 m, in July 2004. A voucher specimen (F 12 250) was deposited at the Herbarium of the Faculty of Sciences and Letters, Yüzüncü Yıl University, Turkey.

Extraction and Isolation. The air-dried, ground, subaerial parts of *S. tomentosa* (398 g) were repeatedly extracted with MeOH (7×1 l) at r.t., 4 d each. After solvent evaporation, 60 g of residue was obtained, which was dissolved in MeOH/H_2O 1:2 (300 ml), and then successively extracted with petroleum ether (PE), AcOEt, and BuOH. The AcOEt-soluble part (12 g) was purified by CC (SiO_2; CH_2Cl_2/MeOH 99:1, 98:2, 95:5, 90:10, 80:20, 70:30, 50:50, 0:100; total volume 6 l): 24 fractions (*Fr. 1–24*). *Fr. 5* (413 mg) was further separated by CC (*Sephadex LH-20*; MeOH): seven fractions (*Fr. 5.1–5.7*). *Fr.*

5.3 (223 mg) was further separated by prep. TLC (SiO_2; CH_2Cl_2/MeOH 9:1) to afford **1** (86 mg) and **5** (33 mg). *Fr. 11* (495 mg) was purified by CC (*Sephadex LH-20*; acetone): 10 fractions (*Fr 11.1–11.10*). *Fr. 11.6* (62 mg) was subjected to semi-prep. HPLC (MeCN/H_2O 25:75; isocratic) to afford **3** (3 mg; t_R 26.8–30.0). *Fr. 12* (631 mg) was purified by CC (*Sephadex LH-20*; MeOH): eleven fractions (*Fr. 12.1–12.11*). *Fr. 12.7* (241 mg) was further separated by semi-prep. HPLC (MeCN/H_2O 22:78; isocratic) to afford **7** (12.7 mg; t_R 21.8–24.8), **8** (17.5 mg; t_R 25.9–29.0), and **3** (1 mg; t_R 37.5–38.0). *Fr. 17* (1.916 g) was subjected to CC (*Sephadex LH-20*; MeOH) to provide 18 subfractions: *Fr. 17.1–17.18*. *Fr. 17 4*, *Fr. 17.8*, and *Fr. 17.14* contained pure **2** (63 mg), **4** (42 mg), and **6** (8 mg), resp.

(±)-Scorzotomentosin (=*(3*RS*)-3,4-Dihydro-3-(4-hydroxyphenyl)-8-methoxy-1*H*-2-benzopyran-1-one*; **1**). Colorless powder. $[\alpha]_D^{25} = 0$ ($c = 2.34$, MeOH). UV (MeOH): 246 (4.1), 306 (3.9). IR (KBr): 3450, 3063, 1736, 1600, 1584, 1475. ^{1}H-, ^{13}C-, and 2D-NMR: see *Tables 1–3*. ESI-MS: 269 ($[M-H]^-$, $C_{16}H_{13}O_4^-$).

*(−)-Scorzotomentosin 4′-*O*-β-Glucoside* (=*4-[(3*S*)-3,4-Dihydro-8-methoxy-1-oxo-1*H*-2-benzopyran-3-yl]phenyl β-Glucopyranoside*; **2**). Colorless powder. $[\alpha]_D^{25} = -142$ ($c = 2.52$, MeOH). CD (MeOH): 241 (pos.), 263 (neg.). ^{1}H-, ^{13}C-, and 2D-NMR: see *Tables 1–3*. HR-FAB-MS: 455.1325 ($[M+Na]^+$, $C_{22}H_{24}NaO_9^+$; calc. 455.1318), 433.1507 ($[M+H]^+$, $C_{22}H_{25}O_9^+$; calc. 433.1499).

(±)-Scorzophthalide (=*(3*RS*)-3-[(*SR*)-Hydroxy(4-hydroxyphenyl)methyl]-7-methoxy-2-benzofuran-1(3*H*)-one*; **3**). Colorless powder. $[\alpha]_D^{25} = 0$ ($c = 2.26$, MeOH). UV (MeOH): 228 (4.2), 285 (3.6), 301 (3.7). IR (KBr): 3440, 1740, 1620, 1607, 1470. ^{1}H-, ^{13}C-, and 2D-NMR: see *Tables 1*, *2*, and *4*. HR-FAB-MS: 287.0912 ($[M+H]^+$, $C_{16}H_{15}O_5^+$; calc. 287.0919).

Scorzoerzincanin (=*2-[(*E*)-2-(4-Hydroxyphenyl)ethenyl]-6-methoxybenzoic Acid*; **4**). Yellowish powder. UV (MeOH): 217 (4.2), 306 (4.31), 320 (4.32). IR (KBr): 3440, 1660, 1590, 1515, 1460, 1400. ^{1}H-, ^{13}C-, and 2D-NMR: see *Tables 1*, *2*, and *4*. ESI-MS: 269 ($[M-H]^-$, $C_{16}H_{13}O_4^-$).

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